# abcam

# Product datasheet

# Anti-BAT3/BAG-6 antibody [EPR9223] ab137076



#### ★★★★★ 2 Abreviews 画像数 11

#### 製品の概要

製品名 Anti-BAT3/BAG-6 antibody [EPR9223]

製品の詳細 Rabbit monoclonal [EPR9223] to BAT3/BAG-6

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), ICC/IF, WB, IHC-P

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide within Human BAT3/BAG-6 (C terminal). The exact sequence is proprietary.

ポジティブ・コントロール HeLa, A431; human fetal brain lysates; Human kidney and testis tissue, mouse and rat brain

tissue lysate. Flow Cyt (intra): HAP1-wt cells.

特記事項 This antibody may not be suitable for IHC with mouse or rat samples.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb patents**.

### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 **EPR9223** 

アイソタイプ lgG

Abpromise保証は、次のテスト済みアプリケーションにおけるab137076の使用に適用されます The Abpromise guarantee アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/30.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 - 1/1000.
ICC/IF		1/500.
WB	*** <u>*</u> (1)	1/1000 - 1/10000. Predicted molecular weight: 119 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

#### ターゲット情報

#### 機能

Chaperone that plays a key role in various processes such as apoptosis, insertion of tailanchored (TA) membrane proteins to the endoplasmic reticulum membrane and regulation of chromatin. Acts in part by regulating stability of proteins and their degradation by the proteasome. Participates in endoplasmic reticulum stress-induced apoptosis via its interaction with AIFM1/AIF by regulating AIFM1/AIF stability and preventing its degradation. Also required during spermatogenesis for synaptonemal complex assembly via its interaction with HSPA2, by inhibiting polyubiquitination and subsequent proteasomal degradation of HSPA2. Required for selective ubiquitin-mediated degradation of defective nascent chain polypeptides by the proteasome. In this context, may play a role in immuno-proteasomes to generate antigenic peptides via targeted degradation, thereby playing a role in antigen presentation in immune response. Key component of the BAG6/BAT3 complex, a cytosolic multiprotein complex involved in the post-translational delivery of tail-anchored (TA) membrane proteins to the endoplasmic reticulum membrane. TA membrane proteins, also named type II transmembrane proteins, contain a single C-terminal transmembrane region. BAG6/BAT3 acts by facilitating TA membrane proteins capture by ASNA1/TRC40: it is recruited to ribosomes synthesizing membrane proteins, interacts with the transmembrane region of newly released TA proteins and transfers them to ASNA1/TRC40 for targeting to the endoplasmic reticulum membrane. Also involved in DNA damage-induced apoptosis: following DNA damage, accumulates in the nucleus and forms a complex with p300/EP300, enhancing p300/EP300-mediated p53/TP53 acetylation leading to increase p53/TP53 transcriptional activity. When nuclear, may also act as a component of some chromatin regulator complex that regulates histone 3 'Lys-4' dimethylation (H3K4me2).

配列類似性

Contains 1 ubiquitin-like domain.

翻訳後修飾

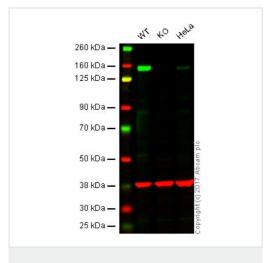
Cleavage by caspase-3 releases a C-terminal peptide that plays a role in ricin-induced apoptosis.

In case of infection by L.pneumophila, ubiquitinated by the SCF(LegU1) complex.

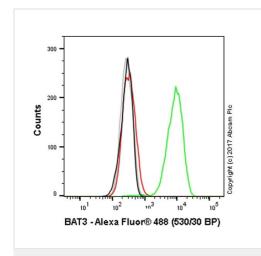
細胞内局在

Cytoplasm > cytosol. Nucleus. The C-terminal fragment generated by caspase-3 is cytoplasmic.

#### 画像



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)



Flow Cytometry (Intracellular) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

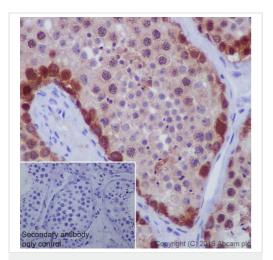
Lane 2: BAT3/BAG-6 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

**Lanes 1 - 3:** Merged signal (red and green). Green - ab137076 observed at 155 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

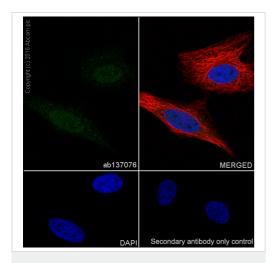
ab137076 was shown to specifically react with BAT3/BAG-6 when BAT3/BAG-6 knockout samples were used. Wild-type and BAT3/BAG-6 knockout samples were subjected to SDS-PAGE. Ab137076 and <u>ab8245</u> (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Overlay histogram showing HAP1 wildtype (green line) and HAP1-BAG6 knockout cells (red line) stained with ab137076. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1 x PBS/10% normal goat serum to block non-specific proteinprotein interactions followed by the antibody (ab137076, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit lgG (H&L) presorbed (ab150081) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-BAG6 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



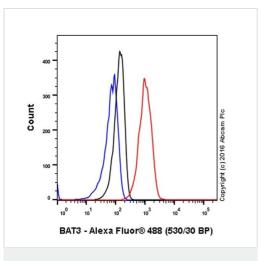
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Immunohistochemical analysis of Paraffin-embedded human testis tissue sections labeling BAT3/BAG-6 with purified ab137076 at a dilution of 1/50 dilution (7.1  $\mu$ g/ml). **ab97051** Goat Anti-Rabbit lgG H&L (HRP) at 1/500 was used as the secondary anitbody. Sections were counterstained with hematoxylin. Antigen retrieval was heat mediated using EDTA Buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



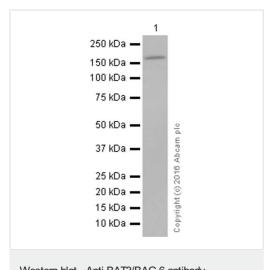
Immunocytochemistry/ Immunofluorescence - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling BAT3/BAG-6 with purified ab137076 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <a href="mailto:ab150077">ab150077</a>, Alexa Fluor<sup>®</sup> 488-conjugated goat antirabbit lgG (1/1000) was used as the secondary antibody. Cells were counter-stained with <a href="mailto:ab195889">ab195889</a> Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) at 1/200. DAPI (blue) was used as a nuclear counterstain. Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Flow Cytometry (Intracellular) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Intracellular Flow Cytometry analysis of HeLa cells labelling BAT3/BAG-6 (red) with purified ab137076 at dilution of 1/30. Goat anti rabbit lgG (Alexa Fluor<sup>®</sup>488) at 1/2000 was used as the secondary antibody. Cells were fixed with 4% paraformaldehyde. Isotype control antibody was Rabbit monoclonal lgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) at 1/5000 dilution (purified) + Mouse brain tissue lysate at 15 µg

#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 119 kDa

Blocking/Diluting buffer 5% NFDM/TBST



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) **All lanes :** Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) at 1/1000 dilution (purified)

**Lane 1 :** A431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2: Human fetal brain tissue lysate

Lane 3: Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Anti-Rabbit  $\lg G$  (HRP), specific to the non-reduced form of  $\lg G$  at 1/2000 dilution

Predicted band size: 119 kDa

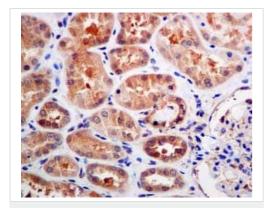
#### Observed band size: 160 kDa

# Blocking/Diluting buffer 5% NFDM/TBST

110 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 488 (525/30 BP) 10<sup>4</sup>

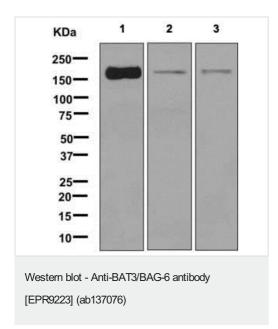
Flow Cytometry (Intracellular) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Overlay histogram showing Hela cells stained with ab137076 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab137076, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor<sup>®</sup> 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labelling BAT3/BAG-6 with unpurified ab137076 at 1/50 dilution.



**All lanes :** Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) at 1/1000 dilution (unpurified)

Lane 1 : HeLa cell lysate Lane 2 : A431 cell lysate

Lane 3: Human fetal brain lysate

Lysates/proteins at 10 µg per lane.

## Secondary

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 119 kDa



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